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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Pia SCHEU et al.
Serial No.	:	09/786,011
For	:	**OLIGONUCLEOTIDES, METHOD AND KIT FOR DETECTING LISTERIA MONOCYTOGENES BY NUCLEIC ACID AMPLIFICATION AND/OR NUCLEIC ACID HYBRIDIZATION**
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Ronald R. Santucci, Reg. No. 28,988
(Name of Applicant, Assignee or Registered Representative)

Signature

September 7, 2004
Date of Signature

TRANSMITTAL OF CERTIFIED ENGLISH TRANSLATION OF GERMAN PRIORITY APPLICATION NO. DE 198 400 44.6

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants have noted that the Examiner has indicated that a certified English translation of the German priority application DE 198 400 44.6 was not submitted in the above application. A certified copy of German priority application DE 198 400 44.6 was provided to the U.S. Patent and Trademark Office by the International Bureau during the international phase of this application. Applicants do not believe there is a further requirement to provide a certified

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English translation of the priority application in order to establish the priority claim, however, in order to ensure that the complete priority information for this application appears on the face of the issued patent, Applicants enclose a certified English translation of DE 198 400 44.6.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: ____________________
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In the matter of:
US 09/786 011

in the name of
Biotecon Diagnostics GmbH

I, Dr. Kai U. Walter, c/o BOETERS & LIECK, Bereiteranger 15, D-81541 München, Germany, do solemnly and sincerely declare as follows:

1. I am fully conversant with the English and German languages.

2. The attached is a translation which I have made in English of DE 198 40 044.6 in the name of Biotecon Diagnostics GmbH which I certify to be a true and correct translation into the English language to the best of my knowledge and belief.

AND I MAKE this solemn declaration, conscientiously believing the same to be true, this 18th day of August 2004.

(Dr. Kai U. Walter)

Oligonucleotides, method and kit for detecting *Listeria monocytogenes* by nucleic acid amplification and/or nucleic acid hybridization

The invention provides oligonucleotides, method and kit for detecting *Listeria monocytogenes* by nucleic acid amplification and/or nucleic acid hybridization.

The genus *Listeria* consists of the six species *L. monocytogenes, L. grayi, L. innocua, L. ivanovii, L. seligeri* and *L. welshimeri.* Among these, only strains of the species *L. monocytogenes* are pathogenic for humans, in particular for those with a weakened immune system and for the elderly and the newborn. The most common symptoms of listeriosis are septicemia, meningitis and miscarriages. *L. monocytogenes* infections are caused especially by consuming contaminated food, in particular milk products, meat, poultry and vegetables.

A large number of methods for detecting *L. monocytogenes* are known. Conventional detection methods for *L. monocytogenes* comprise preconcentrating and subsequently isolating colonies on selection media (Lovett et al., J. Food Protection **50** (1987), 188-192; McClain & Lee, J. Assoc. Off. Anal. Chem. **71** (1988), 660-664). Single colonies are examined for their morphology or for biochemical or serological properties. An analysis may take up to 6 - 8 days.

Since especially readily perishable food is frequently contaminated with *L. monocytogenes*, various high-speed methods for detecting *L. monocytogenes* have been developed. Such methods are based either on immunological methods or on the application of nucleic acid probes.

In this connection, detection may be carried out by direct hybridization of probes to microbe-specific DNA

or RNA (see, for example, Datta, A.R. et al., Appl. Environ. Microbiol. **53** (1987), 2256-2259). The disadvantage of those methods is the low sensitivity, since at least 10^5-10^6 copies of the target nucleic acid are required. This can be compensated by combination with an amplification of the target sequence, for example using the polymerase chain reaction (PCR). A plurality of PCR methods for detecting *L. monocytogenes* have been described in the literature [for a review see, for example, Jones, D.D. & Bej, A.K. in "PCR Technology, Current Innovations", Griffin, H.G & Griffin, A.M., eds., (1994), 341-365]. See also US patents 4,683,195; 4,683,202 and 4,965,188. Furthermore, the ligase chain reaction [WO publication 89/09835], "self-sustained sequence replication" [EP 329,822], "transcription based amplification system" [EP 310, 229] and Qβ RNA replicase system [US patent 4,957,858] may be employed for the amplification of nucleic acids.

Some test kits for detection by means of antibodies are already commercially available. Most of these tests, however, display only low sensitivity and specificity.

To detect specific microorganisms by means of nucleic acid hybridization or nucleic acid amplification, microbe-specific oligonucleotides are commonly used whose base sequence is characteristic for the DNA or RNA of a specific microorganism or of a group of microorganisms. When using said microbe-specific oligonucleotides (for example as primers or probes) in connection with the methods mentioned above, hybridization to the DNA/RNA or amplification of DNA/RNA can occur under suitable reaction conditions only if the DNA/RNA of the particular microorganisms to be detected is present.

The detection methods described for *L. monocytogenes* are based mainly on those target genes which play a

role in the pathogenicity of *L. monocytogenes*. It is known that some of these genes are located on the chromosome next to each other in a virulence gene cluster. Since the listeriolysin gene (hlyA) has been recognized first as to be clearly necessary for the pathogenicity of *L. monocytogenes* (Cossart, P. et al., Infect. Immun. **57** (1989), 3629-3636), most of the genotypic detection methods are based on this gene. The hlyA gene, however, is also found with high homology in nonpathogenic listeria (i.e. in *L. seeligeri* and *L. ivanovii*). In said detection methods, the appearance of false-positive results cannot be completely dismissed, since single point mutations in the region of the binding sites of primers or probes may already be sufficient for this.

It was possible to show that the metalloprotease gene (mpl) which is located in the genome right next to the hlyA gene is only present in *L. monocytogenes*, and thus not in nonpathogenic listeria (Domann, E. et al., Infect. Immun. **59** (1991), 65-72).

The suitability in principle of the DNA region flanking the hlyA gene for detecting *L. monocytogenes* by means of hybridization or amplification has been described (Rossen, L. et al., Int. J. Food Microbiol. **14** (1991), 145-152); however, no oligonucleotide sequences for such detection methods have been published yet.

The sequence of the *L. monocytogenes* mpl gene is described in the EMBL database under accession number X54619 [Domann, E. et al., Infect. Immun. **59** (1991), 65-72]. Furthermore, parts of the sequence of the *L. monocytogenes* mpl gene are listed in the EMBL database under accession number X60035 [Rasmussen, O.F. et al., Infect. Immun. **59** (1991), 3945-3951].

It was an object of the present invention to develop a detection method which is suitable for routine use and

in which the probability of false-positive results appearing is as low as possible for the particular user, even under very variable experimental conditions.

In particular, oligonucleotide sequences are to be provided which can be employed in a detection method for the L. monocytogenes metalloprotease gene (mpl).

These objects are achieved by providing nucleic acid molecules of the sequences

(i) 5'-GAA AAA GCA TTT GAA GCC AT-3' or
(ii) 5'-GCA ACT TCC GGC TCA GC-3' or
(iii) 5'-TCG AAA AAG CAT TTG AAG CC-3' or
(iv) 5'-GGT CAG AGT GAA GCT CAT GT-3' or

(v) of the sequence complementary in each case to (i), (ii), (iii), and (iv).

The oligonucleotides according to the invention may be defined as follows:

Oligonucleotide LM1: (sequence (i) = SEQ ID NO 1 corresponds to the position 2476 to 2495 of *L. monocytogenes* [according to Domann, E. et al. Infect. Immun. **59** (1991), 65-72).
Oligonucleotide LM 2: (sequence (ii) = SEQ ID NO 2) corresponds to the position 2608 to 2624 of *L. monocytogenes*.
Oligonucleotide LM 3: (sequence (iii) = SEQ ID NO 3) corresponds to the position 2474 to 2493 of *L. monocytogenes*
Oligonucleotide LM 4 (sequence (iv) = SEQ ID NO 4) corresponds to the position 2497 to 2516 of *L. monocytogenes*.

In order to investigate the extent to which sequence variations of the mpl gene occur within the species *L.*

monocytogenes, an internal fragment of 300 base pairs of 13 *L. monocytogenes* strains of various serovars (2 strains of the serovars 1/2a, 1 strain of the serovar 1/2b, 1 strain of the serovar 1/2c, 1 strain of the serovar 3a, 1 strain of the serovar 3b, 1 strain of the serovar 3c, 1 strain of the serovar 4a, 1 strain of the serovar 4a/b, 1 strain of the serovar 4b, 1 strain of the serovar 4c, 1 strain of the serovar 4d, and 1 strain of the serovar 7) was sequenced. On the basis of sequence comparisons, it was surprisingly found that the oligonucleotides LM1, LM 2, LM3, LM 4, and also sequences complementary thereto lead to highly specific detections in detection methods for *L. monocytogenes*. The preferred probe in this connection is the oligonucleotide LM4 and the sequences complementary thereto.

The invention in particular provides nucleic acid molecules which are characterized in that, with respect to at least 10 successive nucleotides of their nucleotide chain, they

(a) are identical to 10 successive nucleotides of the above nucleic acid molecules (i) to (v) or
(b) match 9 out of 10 successive nucleotides of the above nucleic acid molecules (i) to (v) or
(c) match 8 out of 10 successive nucleotides of the above nucleic acid molecules (i) to (v) or
(d) are at least 90% homologous to a nucleic acid molecule according to claim 1.

The microbe-specific oligonucleotides are nucleic acids which are from 10 to 250 bases (preferably from 15 to 30 bases) in length.

They may be present in single-stranded or double-stranded form.

Thus, suitable microbe-specific oligonucleotides of the

invention for detecting *L. monocytogenes* are nucleic acids, preferably from 10 to 250 bases and in particular from 15 to 30 bases in length, which match at least in a 10-base sequence the stated sequences LM 1, LM 2, LM 3, LM 4 or the sequences complementary thereto. Relatively small deviations (1 to 2 bases) in this 10-base sequence are possible without the specificity stated in each case being lost during amplification and/or hybridization. It is known to the skilled worker that in the case of such relatively small deviations the reaction conditions have to be modified accordingly; cf., for example, T. Maniatis, Molecular Cloning, G. Sambrook & E.F. Fritsch, editors, Cold Spring Harbour [sic] Laboratory Press, 1989.

To detect *L. monocytogenes*, nucleic acids, preferably genomic DNA, are first released from cells contained in a sample or bacterial culture to be investigated. It is then possible, by means of nucleic acid hybridization and by using the microbe-specific oligonucleotides according to the invention as probe, directly to detect microbe-specific nucleic acid sequences in the sample to be investigated. Various methods known to the skilled worker are suitable for this purpose such as, for example, Southern blot or dot blot.

Especially because of the higher sensitivity, however, an indirect detection method is preferred in which the sought-after DNA/RNA sequences released as described above are first amplified by means of the abovementioned methods for amplifying nucleic acids, preferably PCR.

The primers employed for DNA/RNA amplification using the methods mentioned are the nucleic acid molecules according to the invention. In this connection, specific amplified molecules are formed only if *L. monocytogenes* DNA/RNA is present. A detection reaction (following the amplification reaction) using

the nucleic acid molecules according to the invention as probes can increase the specificity of the detection method. In this subsequent detection reaction, oligonucleotides which are not entirely microbe-specific may likewise be used.

An alternative possibility is for the nucleic acid amplification to be carried out also in the presence of one or more not entirely specific oligonucleotides so that possibly DNA/RNA of other microorganisms not to be detected may also be amplified. An amplification method of this type is usually less specific and should therefore be safeguarded by a subsequent detection reaction using one or more of the nucleic acid molecule(s) according to the invention as probe(s).

According to the invention, it is possible to use various methods in order to detect the amplification products generated in the indirect methods. These include, inter alia, methods known per se such as visualization by means of gel electrophoresis, hybridization of probes to immobilized reaction products [coupled to nylon or nitrocellulose filters (Southern blots) or, for example, to beads or microtiter plates] and hybridization of the reaction products to immobilized probes (for example reverse dot blots or probes coupled to beads or microtiter plates).

According to the invention, there is a large number of possibilities for the oligonucleotides according to the invention (e.g. probes and primers) to be possibly labeled or modified for the direct or indirect detection methods described. Thus, said oligonucleotides may contain, for example, radioactive, colored, fluorescent or otherwise modified or modifying groups, for example antibodies, antigens, enzymes or other substances with affinity to enzymes or enzyme complexes. Probes and primers may be either naturally occurring or synthetically produced double-stranded or

single-stranded DNA or RNA or modified forms of DNA or RNA such as, for example, PNA (in these molecules the sugar units have been exchanged for amino acids or peptides). Individual or a plurality of nucleotides of the probes or primers according to the invention may have been replaced by analogous building blocks (such as, for example, nucleotides which are not naturally present in the target nucleic acid). In particular, up to 20% of at least 10 successive nucleotides of a nucleotide chain, in particular 1 or 2 nucleotides, may have been replaced by analogous building blocks known per se for probes and/or primers.

In the above mentioned indirect detection methods, detection may also involve an internally labeled amplified molecule. This may be carried out, for example, by incorporating modified nucleoside triphosphates (for example coupled to digoxigenin or fluorescein) during the amplification reaction.

The invention further provides a kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*, which kit contains one or more nucleic acid molecules according to the invention.

The nucleic acid molecules according to the invention or the appropriate kits may be used in a method for detecting the presence or absence of bacteria of the species *L. monocytogenes* in a sample, said method being preferably a nucleic acid hybridization and/or a nucleic acid amplification, such as a PCR. In this connection, bacteria to be detected can be distinguished from bacteria not to be detected on the basis of differences in the genomic DNA and/or RNA in at least one nucleotide position in the region of one of the nucleic acid molecules according to the invention.

Example

Example 1: Detection of bacteria of the species *L. monocytogenes* using the polymerase chain reaction

DNA was isolated from pure cultures of the bacteria listed in table 1 by means of standard methods. In each case, approx. 10 to 100 ng of these DNA preparations were then introduced to the PCR in the presence of, in each case, 0.4 μM oligonucleotides LM 1 and LM 2, or LM 3 and LM 2, 200 μM dNTPs (Boehringer Mannheim), 2.5 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 67 mM Tris/HCl (pH 8.8), 0.01% Tween 20 and 0.03 U/μl Taq DNA polymerase (Biomaster). The PCR was carried out in a Perkin Elmer 9600 Thermocycler using the temperature profile listed below:

Initial denaturation	95°C	5 min
35 cycles	94°C	30 sec
	57°C	30 sec
	72°C	30 sec
Final synthesis	72°C	5 min

After finishing the PCR reaction, the amplification products were fractionated by means of agarose gel electrophoresis and visualized by ethidium bromide staining. The expected products of 149 bp and 151 bp in length, respectively, were observed only if DNA of strains of the species *L. monocytogenes* was present. The DNA fractionated in the gels was transferred to nylon filters by means of standard methods and hybridized with the 5' digoxigenin-labeled oligonucleotide LM 4 (sequence 4) in order to test the specificity. Hybridization was carried out in 5 × SSC, 2% blocking reagent, 0.1% lauroylsarcosine, 0.02% SDS and 5 pmol/ml probe at 60°C for 4 h. Washing was carried out using 2 × SSC, 0.1% SDS for 2 × 10 min at

60°C. Detection took place using standard methods by means of alkaline phosphatase conjugates (anti-digoxigenin-AP Fab fragment, Boehringer Mannheim) in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro blue tetrazolium chloride (Boehringer Mannheim).

On the filters, a band was observed only in those cases in which previously a band of 149 bp or 151 bp had been visible on the agarose gel. Thus, the presence of all of the 103 *L. monocytogenes* strains tested was detected by means of PCR and hybridization. In contrast, none of the bacterial strains tested which do not belong to said species were detected by this system.

Table 1: Results of PCR amplification using the oligonucleotides LM 1 and LM 2 (SEQ ID NO 1 and SEQ ID NO 2) and LM 3 and LM 2 (SEQ ID NO 3 and SEQ ID NO 2), respectively, and, in each case, subsequent hybridization using the oligonucleotide LM 4 (SEQ ID NO 4)

Species	Serovar	Strain	LM1/LM2	LM2/LM3
Listeria welshimeri		SLCC 767	-	-
Listeria welshimeri		SLCC 768	-	-
Listeria welshimeri		SLCC 5877	-	-
Listeria welshimeri		SLCC 5828	-	-
Listeria welshimeri		SLCC 6199	-	-
Listeria welshimeri		DSM 20650	-	-
Listeria seeligeri		SLCC 5921	-	-
Listeria seeligeri		SLCC 7303	-	-
Listeria seeligeri		SLCC 7309	-	-
Listeria seeligeri		SLCC 7329	-	-
Listeria seeligeri		SLCC 3954	-	-
Listeria seeligeri		DSM 20751	-	-
Listeria innocua		SLCC 5326	-	-
Listeria innocua		SLCC 7160	-	-
Listeria innocua		SLCC 7161	-	-
Listeria innocua		SLCC 7167	-	-
Listeria innocua		SLCC 7168	-	-
Listeria innocua		DSM 20649	-	-

Listeria innocua		SLCC 3408	-	-
Listeria innocua		NCTC 10528	-	-
Listeria innocua		SLCC 7139	-	-
Listeria grayi		DSM 20601	-	-
Listeria grayi		DSM 20596	-	-
Listeria grayi		BC 7308	-	-
Listeria ivanovii		DSM 20750	-	-
Listeria ivanovii		SLCC 2028	-	-
Listeria ivanovii		SLCC 2098	-	-
Listeria ivanovii		SLCC 2102	-	-
Listeria ivanovii		SLCC 2379	-	-
Listeria ivanovii		SLCC 4121	-	-
Listeria ivanovii		SLCC 4706	-	-
Listeria ivanovii		SLCC 4770	-	-
Listeria ivanovii		SLCC 5378	-	-
Listeria ivanovii		ATCC 19119	-	-
L. monocytogenes		ATCC 19111	+	+
L. monocytogenes		ATCC 19112	+	+
L. monocytogenes		ATCC 19113	+	n.d.
L. monocytogenes		ATCC 19114	+	n.d.
L. monocytogenes		ATCC 19115	+	n.d.
L. monocytogenes		ATCC 19116	+	n.d.
L. monocytogenes		ATCC 19117	+	+
L. monocytogenes		ATCC 19118	+	n.d.
L. monocytogenes		SLCC 53	+	+
L. monocytogenes		SLCC 2479	+	+
L. monocytogenes		SLCC 2482	+	n.d.
L. monocytogenes		SLCC 5835	+	+
L. monocytogenes	1 / 2 a	SLCC 4955	+	+
L. monocytogenes	1 / 2 a	SLCC 6204	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7149	+	+
L. monocytogenes	1 / 2 a	SLCC 7150	+	+
L. monocytogenes	1 / 2 a	SLCC 7153	+	+
L. monocytogenes	1 / 2 a	SLCC 7165	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7195	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7196	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7197	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7198	+	n.d
L. monocytogenes	1 / 2 a	SLCC 7973	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7053	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7054	+	n.d.
L. monocytogenes	1 / 2 a	SLCC 7055	+	n.d.
L. monocytogenes	1 / 2 b	SLCC 6031	+	n.d.
L. monocytogenes	1 / 2 b	SLCC 7163	+	+
L. monocytogenes	1 / 2 b	SLCC 7151	+	+
L. monocytogenes	1 / 2 b	SLCC 7152	+	n.d.
L. monocytogenes	1 / 2 b	SLCC 7354	+	+
L. monocytogenes	1 / 2 b	SLCC 7367	+	n.d.
L. monocytogenes	1 / 2 b	SLCC 7059	+	n.d
L. monocytogenes	1 / 2 c	SLCC 4950	+	+
L. monocytogenes	1 / 2 c	SLCC 6793	+	+
L. monocytogenes	1 / 2 c	SLCC 7154	+	+

L. monocytogenes	1 / 2 c	SLCC 7290	+	n.d.
L. monocytogenes	1 / 2 c	SLCC 7352	+	n.d.
L. monocytogenes	1 / 2 c	SLCC 7355	+	n.d
L. monocytogenes	3 a	SLCC 4949	+	+
L. monocytogenes	3 a	SLCC 7135	+	n.d.
L. monocytogenes	3 a	SLCC 7179	+	n.d.
L. monocytogenes	3 b	SLCC 2540	+	n.d.
L. monocytogenes	3 b	SLCC 7140	+	n.d.
L. monocytogenes	3 b	SLCC 7381	+	n.d
L. monocytogenes	3 c	SLCC 2471	+	+
L. monocytogenes	4 a	SLCC 5069	+	+
L. monocytogenes	4 a	SLCC 5070	+	n.d.
L. monocytogenes	4 a / b	SLCC 7083	+	n.d.
L. monocytogenes	4 a / b	SLCC 7065	+	n.d.
L. monocytogenes	4 a / b	SLCC 7069	+	+
L. monocytogenes	4 b	SLCC 4013	+	+
L. monocytogenes	4 b	SLCC 7194	+	+
L. monocytogenes	4 b	SLCC 7356	+	+
L. monocytogenes	4 b	SLCC 7370	+	+
L. monocytogenes	4 b	SLCC 7372	+	+
L. monocytogenes	4 b	SLCC 7373	+	n.d.
L. monocytogenes	4 b	SLCC 7374	+	n.d.
L. monocytogenes	4 b	SLCC 788	+	n.d.
L. monocytogenes	4 b	SLCC 7056	+	n.d.
L. monocytogenes	4 b	SLCC 7057	+	n.d.
L. monocytogenes	4 b	SLCC 7058	+	n.d.
L. monocytogenes	4 b	SLCC 7060	+	n.d.
L. monocytogenes	4 b	SLCC 7061	+	n.d.
L. monocytogenes	4 b	SLCC 7062	+	n.d.
L. monocytogenes	4 b	SLCC 7063	+	n.d.
L. monocytogenes	4 b	SLCC 7064	+	n.d.
L. monocytogenes	4 b	SLCC 7066	+	n.d.
L. monocytogenes	4 b	SLCC 7067	+	n.d.
L. monocytogenes	4 b	SLCC 7068	+	n.d.
L. monocytogenes	4 b	SLCC 7069	+	n.d.
L. monocytogenes	4 b	SLCC 7070	+	n.d.
L. monocytogenes	4 b	SLCC 7071	+	n.d.
L. monocytogenes	4 b	SLCC 7072	+	n.d.
L. monocytogenes	4 b	SLCC 7073	+	n.d.
L. monocytogenes	4 b	SLCC 7074	+	n.d.
L. monocytogenes	4 b	SLCC 7075	+	n.d.
L. monocytogenes	4 b	SLCC 7076	+	n.d.
L. monocytogenes	4 b	SLCC 7077	+	n.d.
L. monocytogenes	4 b	SLCC 7078	+	n.d.
L. monocytogenes	4 b	SLCC 7079	+	n.d.
L. monocytogenes	4 b	SLCC 7080	+	n.d.
L. monocytogenes	4 b	SLCC 7081	+	n.d.
L. monocytogenes	4 b	SLCC 7082	+	n.d.
L. monocytogenes	4 b	SLCC 7084	+	n.d.
L. monocytogenes	4 b	SLCC 7085	+	n.d.
L. monocytogenes	4 b	SLCC 7086	+	n.d.
L. monocytogenes	4 b	SLCC 7087	+	n.d.

L. monocytogenes	4 b	SLCC 7088	+	n.d.
L. monocytogenes	4 b	SLCC 7089	+	n.d.
L. monocytogenes	4 b	SLCC 7090	+	n.d.
L. monocytogenes	4 b	SLCC 7091	+	n.d.
L. monocytogenes	4 b	SLCC 7092	+	n.d.
L. monocytogenes	4 c	SLCC 4925	+	n.d.
L. monocytogenes	4 c	SLCC 4954	+	+
L. monocytogenes	4 c	SLCC 6277	+	+
L. monocytogenes	4 c	SLCC 6813	+	+
L. monocytogenes	4 c	SLCC 6821	+	+
L. monocytogenes	4 c	SLCC 6823	+	+
L. monocytogenes	4 d	SLCC 2375	+	n.d.
L. monocytogenes	4 d	SLCC 4926	+	+
L. monocytogenes	4 d	SLCC 4952	+	+
L. monocytogenes	7	SLCC 2622	+	+
Arthrobacter spec.		DSM 312	-	n.d.
Bacillus subtilis		ATCC 6051	-	-
Citrobacter freundii		DSM 30040	-	-
Citrobacter koseri		DSM 4595	-	n.d.
Clostridium bifermentans		DSM 630	-	n.d.
Clostridium sporogenes		IfGB 0303	-	n.d.
Enterobacter agglomerans		IfGB 0202	-	n.d.
Enterobacter cloacae		DSM 30054	-	-
Enterobacter gergoviae		BC 674	-	n.d.
Erwinia carotovora		DSM 30168	-	n.d.
Escherichia coli		DSM 30083	-	n.d.
Hafnia alvei		IfGB 0101	-	n.d.
Klebsiella oxytoca		DSM 5175	-	n.d.
Klebsiella pneumoniae		DSM 2026	-	n.d.
Lactobacillus spec.		IfGB 1401	-	n.d.
Lactob. bifermentans		BC 8463	-	-
Leuconostoc carnosum		DSM 5576	-	n.d.
Leucon. mesenteroides		DSM 2146	-	n.d
Micrococcus citreus		IfGB 0601	-	-
Micrococcus luteus		DSM 348	-	-
Pediococcus damnosus		BC 505	-	-
Proteus mirabilis		IfGB 51	-	-
Proteus vulgaris		DSM 2041	-	n.d.
Pseudomonas aeruginosa		ATCC 10145	-	n.d.
Pseudomonas fluorescens		IfGB 0301	-	-
Salmonella spec.		BC 2426	-	n.d.
Salmonella typhimurium		BC 2157	-	n.d.
Serratia marcescens		BC 677	-	-
Shigella flexneri		DSM 4782	-	n.d.
Staphylococcus aureus		ATCC 6538	-	-
Streptococcus faecalis		DSM 20380	-	n.d.
Strept. faecalis		DSM 20478	-	n.d.
Strept. diacetylactis		BC 2149	-	-
Strept. thermophilus		DSM 20259	-	n.d.
Yersinia enterocolitica		DSM 4780	-	n.d.

IfGB: Institut für Gärungsgewerbe Berlin [Institute for Fermentation]

BC: BioteCon Strain Collection

SLCC: H.P.R. Seeliger Listeria Culture Collection, Würzburg, Germany

ATCC: American Type Culture Collection, Rockville, USA

DSM: Deutsche Sammlung von Microorganismen und Zellkulturen GmbH [German Collection of Microorganisms and Cell Cultures],Brunswick, Germany

n.d: not done

Patent claims

1. Nucleic acid molecule

(i) of SEQ ID NO 1 5'-GAA AAA GCA TTT GAA GCC AT-3' or
(ii) of SEQ ID NO 2 5'-GCA ACT TCC GGC TCA GC-3' or
(iii) of SEQ ID NO 3 5'-TCG AAA AAG CAT TTG AAG CC-3' or
(iv) of SEQ ID NO 4 5'-GGT CAG AGT GAA GCT CAT GT-3' or
(v) of the sequence complementary in each case to (i), (ii), (iii), and (iv).

2. Nucleic acid molecule, **characterized in that**, with respect to at least 10 successive nucleotides of its nucleotide chain, it

(i) is identical to 10 successive nucleotides of the nucleic acid molecules according to claim 1 or
(ii) matches 9 out of 10 successive nucleotides of the nucleic acid molecules according to claim 1 or
(iii) matches 8 out of 10 successive nucleotides of the nucleic acid molecules according to claim 1 or
(iv) is at least 90% homologous to a nucleic acid molecule according to claim 1.

3. Nucleic acid molecule according to claim 2, **characterized by** a length of from 10 to 250 and preferably of from 15 to 30 nucleotides.

4. Nucleic acid molecule according to any of the preceding claims, **characterized in that** it is present in single-stranded or double-stranded form.

5. Nucleic acid molecule according to any of the

preceding claims, **characterized in that** it is present

(i) as DNA sequence or
(ii) as RNA sequence corresponding to (i) or
(iii) as PNA sequence,

where the nucleic acid molecule is modified, where appropriate, in a manner known per se for analytical detection methods, in particular for those based on hybridization and/or amplification.

6. Nucleic acid molecule according to any of the preceding claims, **characterized in that** up to 20% of at least 10 successive nucleotides of its nucleotide chain, in particular 1 or 2 nucleotides, have been replaced by analogous building blocks known per se for probes and/or primers, in particular by nucleotides not naturally present in bacteria.

7. Nucleic acid molecule according to any of the preceding claims, **characterized in that** the nucleic acid molecule has been modified or labeled by or additionally by having one or more radioactive groups, colored groups, fluorescent groups, groups for immobilization on a solid phase and/or groups for an indirect or direct reaction, in particular for an enzymatic reaction, in particular with the aid of antibodies, antigens, enzymes and/or substances with affinity to enzymes or enzyme complexes, and/or otherwise modifying or modified groups of a nucleic acid-like structure.

8. Kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*, **characterized by** one or more nucleic acid molecules according to any of the preceding claims.

9. Use of one or more nucleic acid molecules

according to any of claims 1 to 7 or of a kit according to claim 8 for detecting the presence or absence of bacteria of the species *Listeria monocytogenes*.

10. Use according to claim 9, **characterized in that** a nucleic acid hybridization and/or a nucleic acid amplification are carried out.

11. Use according to claim 10, **characterized in that** for the nucleic acid amplification a polymerase chain reaction is carried out.

12. Use according to claim 1, **characterized in that** the bacteria to be detected are distinguished from the bacteria not to be detected on the basis of differences in the genomic DNA and/or RNA in at least one nucleotide position in the region of one of the nucleic acid molecules according to claim 1.

Abstract

The present invention relates to a nucleic acid molecule or nucleic acid molecules and also to a method for the rapid and sensitive detection of bacteria of the pathogenic species *Listeria monocytogenes*. The invention further relates to a test kit or test kits for carrying out the detection methods mentioned.